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Project title: Protein kinases in metabolic regulation in *Mycobacterium tuberculosis*

Beneficiary: Dr Helen O'Hare at the University of Leicester, UK.

hmo7@le.ac.uk +44 116252 2947

In 2010 there were 9 million new cases of tuberculosis and 1.4 million deaths from tuberculosis (World Health Organisation report 2011). Globally, research into the causative agent of TB, *Mycobacterium tuberculosis*, is important in understanding the process by which this pathogen causes disease and identifying routes to generate new treatments for the disease. The specific topic of this project was signalling by protein kinases in *Mycobacterium tuberculosis*. Some of these kinases are thought to be importance in survival (serine threonine protein kinase B, PknB) and virulence (serine threonine protein kinase G, PknG) of *M. tuberculosis*.

The first objective of the project was to determine the function of a protein named GarA, which is a substrate of both kinases PknB and PknG. We have shown that GarA controls energy and amino acid metabolism by binding to three enzymes and directly modulating their activities. These enzymes are alpha-ketoglutarate decarboxylase, glutamate dehydrogenase and glutamate synthase (O'Hare et al, Nott et al, Wagner et al), and they catalyse central steps in the citric acid cycle and glutamate metabolism. In turn, the kinases control GarA by switching it off via phosphorylation.

The second objective was to investigate regulation of GarA phosphorylation and regulation of kinase activity. This was an ambitious goal, since little is known about what signals activate bacterial serine threonine protein kinases. Towards this aim, we have shown that although the amount (concentration) of GarA in *M. tuberculosis* is constant, the percentage of GarA that is phosphorylated varies widely depending on the available nutrients and the phase of growth (rapid growth or stationary phase). This confirms the role of GarA as a regulator and gives the first hints at the signals that might trigger phosphorylation during rapid growth and dephosphorylation during stationary phase. This work is being prepared for publication.

The final objective was to investigate the effects of protein kinases and GarA on growth and virulence of *M. tuberculosis*. This has been done by culturing *M. tuberculosis* and related non-pathogenic mycobacteria that have disruptions in *pknG* and *garA* genes. In contrast to previous studies on the vaccine strain *M. bovis* BCG, we have found that *M. tuberculosis* H37Rv does not require a functional copy of the *pknG* gene for survival inside cultured human macrophages (unpublished). In contrast, disruption of *garA* changed the morphology and *in vitro* growth characteristics of non-pathogenic *M. smegmatis*.

This work calls into question the suitability of PknG as a target for anti-tuberculosis drug development, since strains lacking PknG can grow *in vitro* and infect human macrophages. However, we have provided evidence of the importance of serine threonine protein kinases in *M. tuberculosis*, since protein phosphorylation regulates three key steps in central metabolism, and disruption of this processes causes defects

in growth and survival. This will impact on the understanding of bacterial signalling in general, and current and future research targeting other protein kinases for anti-tuberculosis drug development.

O'Hare HM, Duran R, Cervenansky C, Bellinzoni M, Wehenkel A, Pritsch O, Obal G, Baumgartner J, Vialeret J, Johnsson K, Alzari PN (2008) Regulation of glutamate metabolism by protein kinase G in mycobacteria. *Mol Microbiol*. **70** (6) 1408-1423.

Nott TJ, Kelly G, Stach L, Li J, Westcott S, Patel D, Hunt DM, Howell S, Buxton RS, O'Hare HM, Smerdon SJ (2009) An intra-molecular switch regulates phospho-independent FHA domain interactions in *Mycobacterium tuberculosis*. *Science Signaling* 2 (63) ra12.

Wagner T, Bellinzoni M, Wehenkel A, O'Hare HM, Alzari PM (2011) Functional plasticity and allosteric regulation of alpha-ketoglutarate decarboxylase in central mycobacterial metabolism. Chemistry & Biology 18 (8) 1011-1020.